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Deficient Growth of C57BL Marrow Cells Transplanted in F_1 Hybrid Mice

ASSOCIATION WITH THE HISTOCOMPATIBILITY-2 LOCUS

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Summary. The ability of C57BL, C3H, and A strain marrow cells to proliferate on transplantation into irradiated isogenic, F_1 hybrid, and backcross progeny mice has been investigated by the spleen-colony technique and by measuring the newly-formed DNA in the recipient spleen with [^{131}I] 5-iododeoxyuridine. Transplants of C57BL cells grew poorly in $(A \times C57BL)F_1$ and in $(C57BL \times C3H)F_1$ and reciprocal hybrids, as compared with isogenic and allogeneic hosts, whereas C3H and A strain marrow grafts were successful in isogenic, F_1 hybrid and backcross recipients. In segregating backcross progeny, i.e. in offspring from F_1 hybrid females mated to C57BL males, the frequency of success or failure of the C57BL grafts suggested that the trait was controlled by a single pair of genetic determinants at an autosomal locus. The latter is apparently linked with, or part of, the H-2 region in the IXth linkage group. The experimental evidence suggested also that the failure of C57BL haemopoietic cell grafts in H-2 heterozygotes was not related to exhaustion of donor cells by excess of recipient isoantigen but rather to lack of expression in the heterozygotes of histocompatibility-related growth requirements yet undefined. These requirements are specific for haemopoietic cells, but not for skin grafts, and resulted most probably from the effect of genetic (interallelic) interaction involving H-2 or an H-2-linked locus.

INTRODUCTION

The success or failure of allogeneic† tissue grafts in the mouse depends on whether donor and recipient animal share a number of independent histocompatibility (H) genes (Little, 1941; Snell, 1958; Snell and Stevens, 1961). These genes determine the specificity of certain cellular antigens (Gorer, 1937; Amos, 1962); there is agreement, however, that non-immunological modes of action of the H genes, although not recognized so far, cannot be ruled out (Hauschka and Furth, 1957; Snell, 1958; Stimpfling and Snell, 1962).

Our present understanding of the genetic basis of tissue transplantation is based on experiments on grafting of tumour tissue or skin in mice. Under these circumstances, grafts from either inbred homozygous parent are generally accepted by F_1 hybrids, and F_1 hybrid tissues are uniformly rejected by inbred parental-strain recipients. Both patterns

* Operated by Union Carbide Corporation for the U.S. Atomic Energy Commission.

† The nomenclature is used according to Gorer, Loutit and Micklem (1961).

of response are interpreted as evidence that the F_1 hybrid progeny from crosses between two parental strains of mice inherit all the parental H genes and the related antigenic specificities. Further attempts to detect genetic interaction in the inheritance and/or expression of such cellular antigens, with the use of serological techniques (Stimpfling and Snell, 1962) as well as solid tissue transplantation (Martinez, Shapiro and Good, 1959; Wigzell and Linder, 1961), have been unsuccessful in the mouse, although the occurrence of such interactions is well-documented in several other species and species hybrids (see review articles by Owen (1960) and by Stimpfling and Snell (1962)).

At variance with the behaviour of solid tissue grafts in mice were the observations made by Snell (1958), Boyse (1959) and Snell and Stevens (1961) that leukaemia cells and antibody-forming spleen cells of C57BL mice do not grow on transplantation into F_1 hybrids as well as in isogenic hosts. The inhibition of growth was found to be exaggerated in spleen cells from C57BL donors immunized against isoantigens of the second parental strain, a result suggesting that such cells were hypersensitive toward the isoantigens in the hybrid. The reduction of growth was attributed to exhaustion of the transplanted cells through a graft-versus-host reaction initiated by isoantigens in the hybrid. This interpretation implies, however, that C57BL immune cells were present and in a majority both in spleen and leukaemic grafts, and that the immune cells of the C57BL strain were more sensitive to continuous isoantigenic stimulation than those of other strains.

The present study was an outgrowth of earlier experiments (Cudkowicz, 1961) and was undertaken to explore whether bone marrow cells of C57BL donors which presumably would not be engaged in antibody production would grow poorly in F_1 hybrids. It was also the purpose of this study to establish whether or not deficient growth of C57BL haemopoietic cells in F_1 hybrids resulted from the effect of gene interaction or of recessive genetic determinants not expressed in the F_1 progeny from crosses between C57BL and other inbred mouse strains.

MATERIALS AND METHODS

Design of Experiments

Bone marrow cells injected into a heavily X-irradiated animal become established as a haemopoietic transplant even when host and donor differ at major histocompatibility loci (Koller, Davies and Doak (1961), a review). In this study, the repopulation of irradiated recipients by known numbers of grafted marrow cells was assessed quantitatively at given time intervals by counting the number of nodular haemopoietic colonies in the spleen derived from the proliferating donor stem cells, according to the method of Till and McCulloch (1961). Repopulation was also assessed by measuring the splenic uptake of the thymidine analogue, ^{131}I-labelled 5-iododeoxyuridine ([^{131}I] UdR), a specific deoxyribonucleic acid (DNA) precursor, by the method of Cudkowicz, Upton, Smith, Gosslee and Hughes (1964). Parental bone marrow cells from several donors were pooled in a single preparation for infusion into recipient mice of different genotypes. In this way the results could be evaluated by comparing data within an experiment as well as between experiments involving different cell preparations, since marked variations in the proliferative capacity of marrow were observed between individual donor mice. A schematic outline of these procedures is presented in Fig. 1.

First we assessed the proliferative activity of C57BL marrow cells grafted into isogenic, F_1 hybrid and allogeneic hosts. The effect of variables such as dose of X rays to recipient

mice, size of donor cell inoculum, and length of time allowed for growth were also investigated. The proliferation of C3H and C57BL cells was compared and the fate of C57BL skin grafts was studied under conditions in which the C57BL marrow cells failed to grow.

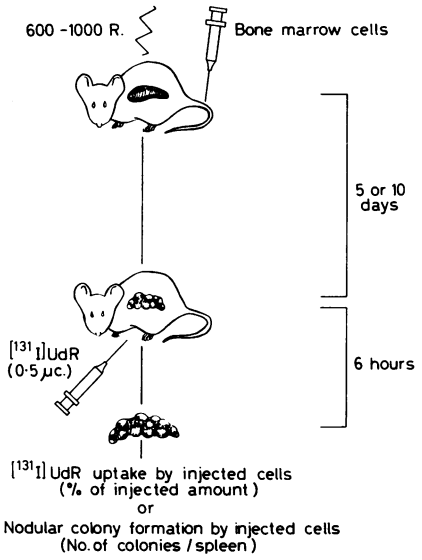

FIG. 1. Schematic outline of experimental procedures (see text).

Second, C57BL, C3H and A strain marrow cells were grafted into backcross progeny mice obtained by mating (C3H × C57BL)F_1 and (A × C57BL)F_1 females to C57BL and to C3H or A strain males, respectively. The backcross progeny were individually identified and classified for susceptibility to parental marrow cell grafts, for sex, and for the marker genes histocompatibility-2 (H-2), agouti (A), and haemoglobin (Hb) of the IXth, Vth and Ist linkage groups, respectively.

Animals

Adult mice of the following strains and their F_1 hybrids were used: C57BL/10ScSn (B/10), C3H/DiSn (C3H) and A/Sn (A). Pedigreed breeding pairs were supplied by Dr. G. D. Snell during 1960–1963 and maintained by brother–sister mating thereafter at Oak Ridge. F_1 hybrid mice were backcrossed at the Roscoe B. Jackson Memorial Laboratory and their offspring were shipped to Oak Ridge shortly before their use in these experiments. Inbred C3H/Anf and C57BL (BL) mice were also used in some instances; they were purchased from Cumberland View Farms, Clinton, Tennessee, at the age of 5 weeks. The symbols in parentheses are abbreviations used in this paper, whereas C57BL indicates mice of this strain, irrespective of the subline. All the animals were housed five to ten to a cage in temperature–humidity controlled quarters. F_1 hybrid and backcross-progeny mice are designated by listing first the female and then the male parental strain; e.g. (B/10♀ × C3H♂)F_1, [(C3H♀ × B/10♂)F_1♀ × B/10♂].

Irradiation

An X-ray machine was used, with the following factors: 300 kVp., 20 mA., 4·75 mm. Be inherent filtration and 3 mm. Al added filtration, 93·5 cm. target-to-mouse distance,

hvl. of ~0·5 mm. of Cu. The exposure rate, in air, was 80 R./min. The mice were irradiated in a partitioned, revolving, Perspex exposure cage.

Bone Marrow Transplantation

Donor mice of the same sex as the recipients were killed by exsanguination, and bone marrow cells were obtained from femurs and tibias. The cells were suspended in cold Tyrode's solution and passed through a 200-mesh stainless steel screen. Total nucleated cell counts and counts of eosin-stained cells were made by haemocytometer. The proportion of the latter cells was found consistently to be 10–15 per cent. The concentration of eosin-unstained cells was adjusted immediately thereafter to the desired level, and cells were infused by tail vein into recipient mice, generally within 2 hours after the donor was killed.

Skin Grafts

Parental skin was grafted onto F_1 hybrid recipients by placing the graft upon the *panniculus carnosus* of the mid-dorsal region. The donor skin was taken from the ears by cutting them at their insertion and by separating the two skin layers with small forceps. The cartilage was not scraped from the grafts. Bandages were removed from recipient mice 7 days after the graft was applied. Gross inspection was performed daily thereafter in the first month, weekly during the second and third month, and every second week thereafter until 6 months.

Estimation of Marrow Colony-Forming Activity

The method used is fully described elsewhere (Till and McCulloch, 1961). Graded doses of donor cells were injected into groups of isogenic and F_1 hybrid recipients exposed to 700 R. of whole-body X-irradiation. Ten days later their spleens were removed, fixed in Bouin's fluid, and examined with the naked eye to count visible nodular colonies. In each experiment, the number of endogenous spleen colonies in irradiated animals not given marrow was also determined and was found to be not larger than one colony per ten spleens. Standard errors were calculated on the mean number of colonies per spleen.

Estimation of [^{131}I] UdR Uptake by Transplanted Marrow

The use of [^{131}I] UdR for quantitative studies of cellular proliferation was proposed by Gitlin, Commerford, Amsterdam and Hughes (1961) and has proved accurate and reproducible for estimation of growth of bone marrow cells *in vivo* (Cudkowicz *et al.*, 1964). Graded doses of marrow were infused into groups of X-irradiated recipient mice. Generally, each cell dose was given to at least five mice, and five cell doses were employed to obtain cell dose [^{131}I] UdR uptake response curves. Five days after marrow injection, unless otherwise stated, each recipient mouse received intraperitoneally 0·5 μc. of carrier-free [^{131}I] UdR (of the order of one curie per micromole; synthesized and kindly supplied by W. L. Hughes, Medical Research Center, Brookhaven National Laboratory) to label the DNA newly synthesized in the spleen by the transplanted cells. No stable iodine was given beforehand to saturate the thyroid gland. Spleens were not removed until 6 hours later to allow excretion of non-incorporated ^{131}I radioactivity. Each whole spleen was placed in a glass test tube and its radioactivity was measured in a 2×2 in. well-type scintillation counter. With each experiment, the spleen ^{131}I activity of irradiated

—not cell-injected—mice (radiation controls) was also determined to enable correction of gross incorporation values for host, as opposed to donor, contributions. The [^{131}I] UdR content of the spleen was expressed as a percentage of the total radioactivity injected into the animal: it resulted almost entirely from uptake of the compound into DNA of proliferating donor cells. Indeed, the host contribution, as measured in mice exposed to 700 R. of X-rays, never exceeded an uptake value of 0·05 per cent of the injected [^{131}I] UdR. Uptake values of repopulated spleens were in the range of 0·1–1 per cent.

Tests of significance were made using logarithms of the [^{131}I] UdR uptake values to stabilize the variance. When regression analysis was indicated, straight lines were fitted by the method of least squares.

Determination of H-2 Serotype

Backcross progeny mice were serotyped by the polyvinylpyrrolidone haemagglutination technique, as described by Stimpfling (1961).

Determination of Haemoglobin Type

Solubility and crystallographic properties of peripheral blood haemoglobin have been determined by the method of Popp and Cosgrove (1959). The haemotypes of several strains of mice, including C57BL, C3H and A mice, have been characterized by Popp (1963).

RESULTS

TRANSPLANTATION OF BL, B/10, C3H/Anf, AND C3H MARROW CELLS INTO ISOGENIC, F_1 HYBRID AND ALLOGENEIC HOSTS

The relation between the number of transplanted donor cells and (1) the mean number of colonies and (2) the mean uptake of [^{131}I] UdR in the repopulated spleens of isogenic recipients is shown in Table 1 and Fig. 2 respectively. In both instances, the extent of spleen repopulation was a linear function of cell dose, but for colonies the range of cell

TABLE 1

THE RELATIONSHIP BETWEEN THE NUMBER OF TRANSPLANTED BL OR C3H/Anf MARROW CELLS AND THE NUMBER OF COLONIES FORMED IN THE SPLEEN OF ISOGENIC AND F_1 HYBRID RECIPIENTS EXPOSED TO 700 R. OF WHOLE-BODY X-IRRADIATION

Donor		*Recipients**		
Strain	*No. of cells* ($\times 10^5$)	*BL* ♀	*(C3H/Anf × BL)F$_1$* ♀	*C3H/Anf* ♀
BL ♀	0·2	3·7 ± 0·5 (17)†		
	0·4	6·4 ± 0·4 (35)	0·3 ± 0·1 (40)	
	7·5	>20 (20)	0·4 ± 0·1 (39)	
	15		2·6 ± 0·4 (40)	
	20		5·8 ± 0·5 (20)	
	30		12·4 ± 1·3 (10)	
C3H/Anf ♀	0·2		3·7 ± 0·3 (29)	4·2 ± 0·4 (29)
	0·3		4·6 ± 0·2 (69)	5·6 ± 0·2 (38)
	0·4		6·7 ± 0·6 (8)	7·8 ± 0·8 (10)

* Mean number of colonies per spleen ± S.E.M. (standard error of the mean).
† Number of mice in parentheses.

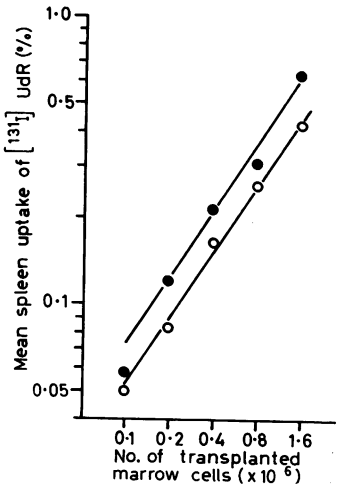

FIG. 2. Mean spleen uptake of [^{131}I] UdR in relation to the number of bone marrow cells injected into X-irradiated (700 R.) isogenic recipient mice (five mice per point). ● = B/10 cells, slope of regression and 95 per cent confidence limits 0·83 (0·75, 0·87); ○ = C3H cells, slope of regression and 95 per cent confidence limits 0·88 (0·83, 0·92).

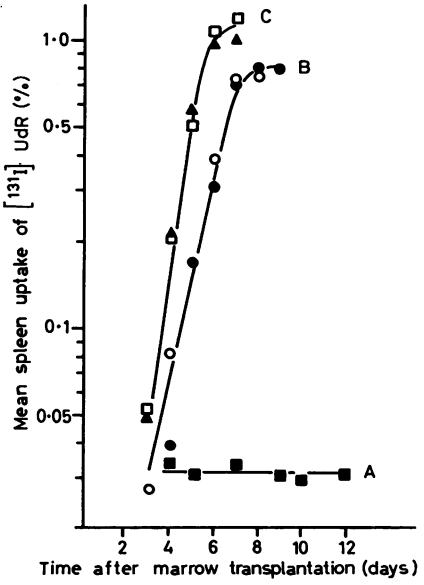

FIG. 3. Mean spleen uptake of [^{131}I] UdR in relation to time after marrow cell transplantation into X-irradiated recipient mice (five to seven mice per point).
● $0{\cdot}50 \times 10^6$ B/10 cells → B/10, 800 R. recipients. Slope of regression ± standard error = $0{\cdot}39 \pm 0{\cdot}09$.
■ 1×10^6 B/10 cells → (B/10 × C3H)F_1, 900 R. recipients.
▲ 10×10^6 B/10 cells → (B/10 × C3H)F_1, 900 R. recipients. Slope of regression ± standard error = $0{\cdot}51 \pm 0{\cdot}08$.
○ $0{\cdot}50 \times 10^6$ C3H cells → C3H, 800 R. recipients. Slope of regression ± standard error = $0{\cdot}38 \pm 0{\cdot}08$.
□ 1×10^6 C3H cells → (B/10 × C3H)F_1, 900 R. recipients. Slope of regression ± standard error = $0{\cdot}46 \pm 0{\cdot}05$.

doses yielding linear responses was rather narrow. Coalescence of colonies arising from crowding occurred consistently in spleens of mice given more than 8×10^4 marrow cells, thereby preventing accurate determination of the response to the larger marrow inocula. On the other hand, the linear portion of the [^{131}I] UdR uptake response was considerably broader, extending from 10^5 to $\sim 2 \times 10^6$ transplanted marrow cells, probably because crowding of proliferating donor cells in recipient spleens interfered neither with incorporation by competent cells of radioactive DNA precursor nor with the counting efficiency of the incorporated radioactivity. The data show that BL and B/10 marrow cells, on the one hand, and C3H/Anf and C3H marrow cells, on the other, were equally effective in repopulating the spleens of isogenic recipients, irrespective of whether their proliferative activity was assessed at 5 (Fig. 2) or 10 days (Table 1) after transplantation.

Proliferation of B/10 and C3H marrow cells was also assessed at varying intervals after their infusion into isogenic hosts to determine whether the two cell types would grow at comparable rates in the repopulated spleens. The results (Fig. 3, curve B) indicate that growth of the transplanted cells was exponential over a certain length of time and that the slopes of the growth curves were identical for comparable numbers of transplanted donor cells. Hence, under conditions of lack of histoincompatibility no inherent differences of growth potential or growth pattern were detected among marrow cell populations carrying the B/10 or the C3H genotype.

The two cell types, however, differed markedly in their ability to repopulate the recipient spleen when infused into hybrid hosts derived from crosses between inbred BL and C3H/Anf, or B/10 and C3H parents (Tables 1 and 2 and Fig. 3, curves A and C). Although the C3H/Anf and C3H marrow cells promoted comparable colony formation and [^{131}I] UdR uptake in spleens of isogenic and hybrid recipients, the BL and B/10 cells failed almost completely to produce colonies and [^{131}I] UdR uptake in spleens of hybrids at the doses of transplanted cells yielding a linear response or exponential growth pattern in spleens of isogenic BL and B/10 mice. The failure of these marrow cells to grow persisted until radiation-induced death of the hybrid recipient (Fig. 3, curve A). Reciprocal hybrids, i.e. (C3H/Anf $\times$ BL)F_1 and (C3H $\times$ B/10)F_1 as compared with (B/10 $\times$ C3H)F_1, were equally 'resistant' to the parental BL and B/10 marrow grafts, irrespective of the sex of the donor and recipient (Tables 1 and 2). 'Hybrid resistance' or 'resistant' are descriptive terms used hereafter to indicate that parental marrow cell grafts sufficient for repopulation of irradiated isogenic recipients are not adequate for repopulation of similarly irradiated hybrid recipients. The 'resistance' so defined could be overridden by increasing the number of inoculated BL or B/10 cells; i.e. when the parental cells were allowed to grow in the recipients for 5 days, several multiples of the cell dose inoculated into isogenic mice were necessary to obtain comparable spleen repopulation in hybrid mice (Table 2 and Fig. 3, curve C).

B/10 marrow cells were infused into B/10, (B/10 $\times$ C3H)F_1 and C3H mice irradiated with graded exposures of whole-body X-rays to determine whether the 'hybrid resistance' toward B/10 cell grafts was radiosensitive, and if so, how it would compare with the resistance of a C3H host capable of a homograft reaction against B/10 cellular isoantigens (Table 3). Maximal proliferation of donor cells was observed in isogenic recipients whenever the X-ray exposure exceeded 600 R. It was inferred, therefore, that sublethal as well as lethal exposures to ionizing radiation created optimal conditions for proliferation of injected donor haemopoietic cells, and that under circumstances characterized by lack of host–donor histoincompatibility the [^{131}I] UdR uptake of the recipient's spleen

TABLE 2

UPTAKE OF [^{131}I] UdR IN THE SPLEEN OF ISOGENIC AND F_1 HYBRID RECIPIENT MICE GRAFTED WITH B/10 OR C3H MARROW CELLS

Donor			*Recipients**											
			Isogenic with donor				$(B/10 \times C3H)F_1$				$(C3H \times B/10)F_1$			
Strain	*Sex*	*No. of cells* ($\times 10^6$)	♀		♂		♀		♂		♀		♂	
B/10	♀	1	0·27 ± 0·03	(9)†	0·31 ± 0·04	(10)	0·05 ± 0·008	(14)	0·01 ± 0·001	(8)	0·03 ± 0·001	(10)	—	
		10	—		—		0·39 ± 0·04	(15)	0·38 ± 0·06	(9)	0·41 ± 0·08	(5)	—	
B/10	♂	1	0·26 ± 0·04	(10)	0·29 ± 0·03	(10)	0·04 ± 0·002	(10)	0·05 ± 0·004	(10)	—		0·04 ± 0·001	(10)
		10	—		—		0·22 ± 0·05	(9)	0·28 ± 0·06	(10)	—		—	
C3H	♀	1	0·30 ± 0·04	(6)	0·38 ± 0·03	(6)	0·30 ± 0·05	(10)	0·34 ± 0·04	(10)	0·31 ± 0·05	(10)	—	

* Exposed to 700 R. of X-rays before grafting.
† Mean uptake per spleen ± standard error of the mean; number of mice in parentheses.

TABLE 3

UPTAKE OF [^{131}I] UdR IN THE SPLEEN OF ISOGENIC, ALLOGENEIC AND F_1 HYBRID MICE IN RELATION TO X-RAY EXPOSURE AND TO INFUSION OF B/10 MARROW CELLS

No of donor cells ($\times 10^6$)	*Recipient strain**	*X-ray exposure (R.)*				
		600	700	800	900	1000
1	B/10	0·35 (0·28, 0·45)†	0·30 (0·25, 0·37)	0·32 (0·26, 0·39)	0·31 (0·23, 0·42)	0·28 (0·23, 0·35)
1	C3H	0·17 (0·15, 0·20)	0·17 (0·13, 0·21)	0·21 (0·15, 0·31)	0·28 (0·22, 0·37)	0·30 (0.24, 0·38)
1	$(B/10 \times C3H)F_1$	0·02 (0·00, 0·03)	0·02 (0·01, 0·04)	—	0·01 (0·00, 0·03)	0·05 (0·02, 0·09)
10	$(B/10 \times C3H)F_1$	0·08 (0·07, 0·10)	0·15 (0·08, 0·27)	0·26 (0·20, 0·34)	0·27 (0·15, 0·48)	0·51 (0·39, 0·66)

* Seven to nine mice per group.
† Mean uptake per spleen; 95 per cent confidence limits in parentheses.

depended exclusively on the number of competent marrow stem cells injected. B/10 cells from the same marrow preparation, but infused into C3H mice, also grew optimally, as judged from [^{131}I] UdR spleen uptake, in recipients exposed to more than 800 R. of X-rays. However, growth was suboptimal in C3H recipients at lower exposures. Repopulation by B/10 marrow cells in hybrid recipients remained suboptimal or absent, depending on the size of the cell inoculum, at all tested exposure levels, up to 1000 R. of X-rays. Nevertheless, a moderate degree of radiosensitivity of the 'hybrid resistance' to B/10 cells was indicated by the increase in spleen uptake of [^{131}I] UdR with increased X-ray exposure in the range from 600 to 1000 R. of X-rays.

TRANSPLANTATION OF B/10 SKIN ONTO ISOGENIC AND F_1 HYBRID RECIPIENTS

A total of twenty mice of each type was grafted with B/10 skin. The grafting was simultaneous with some of the experiments involving marrow cell transfer, and in those instances the F_1 hybrid skin recipients were littermates or siblings of the marrow recipients. All skin grafts were successful and persisted without changes for 6 months.

TABLE 4

MEAN UPTAKE OF [^{131}I] UdR IN THE SPLEEN OF ISOGENIC, F_1 HYBRID AND BACKCROSS PROGENY MICE GRAFTED WITH 10^6 PARENTAL MARROW CELLS

Donor strain	*Recipient**			*Uptake* (%) ± *S.E.M.*†
	Strain	*Sex*	*No. of animals*	
B/10 ♀	B/10	♀	4	0·23 ±0·01
	B/10	♂	5	0·24 ±0·02
	B/10	♀+♂	11	0·31 ±0·04
	(C3H ×B/10)F_1	♀	5	0·007 ±0·002
	(A ×B/10)F_1	♀+♂	9	0·02 ±0·01
	(C3H ×B/10)F_1 ×B/10‡	♀+♂	21	0·24 ±0·02
		♀+♂	24	0·02 ±0·003
	(A ×B/10)F_1 ×B/10	♀+♂	12	0·40 ±0·04
		♀+♂	13	0·005 ±0·005
C3H ♀	C3H	♀	4	0·29 ±0·03
	C3H	♂	5	0·23 ±0·03
	(C3H ×B/10)F_1	♂	5	0·23 ±0·03
	(C3H ×B/10)F_1 ×C3H	♀+♂	40	0·33 ±0·02
A ♀	A	♀	6	0·57 ±0·05
	(A ×B/10)F_1	♀+♂	9	0·45 ±0·06
	(A ×B/10)F_1 ×A	♀+♂	32	0·57 ±0·03

* Recipients exposed to 700 R. of X-rays.
† Standard error of the mean.
‡ Data of two independent experiments were pooled.

TRANSPLANTATION OF PARENTAL B/10, C3H AND A MARROW CELLS INTO BACKCROSS-PROGENY MICE

The observation that male and female F_1 hybrid mice produced by reciprocal crosses were equally 'resistant' to transplants of 10^6 parental B/10 marrow cells suggested the possible existence of an autosomal genetic factor(s) in the B/10 line, not expressed in F_1 hybrids, influencing the growth of transplanted parental haemopoietic cells. The progeny of such hybrid females backcrossed to males of either parental line were classified as to

'resistance' or susceptibility to marrow grafts of their respective parents by determining spleen uptake of [^{131}I] UdR 5 days after exposure to 700 R. and infusion of 10^6 cells. The distinction between the two phenotypes, as determined by the splenic [^{131}I] UdR uptake values, was usually clear, and no overlapping owing to individual fluctuations occurred. The backcross mice were 12–25 weeks old and probably heterogeneous in respect to radiosensitivity; however, recipient spleen repopulation by 10^6 B/10 donor cells was known to be dependent neither on radiation in the range of 600–1000 R. of whole-body exposure (Table 3, first and third line of data section) nor on the number of days elapsed from cell infusion into the heterozygotes (Fig. 3, curve A). Independent segregation of other H genes in H-2 homozygous backcross mice also did not influence the extent of repopulation by transplanted parental marrow cells, provided the recipients were irradiated and not presensitized against parental isoantigens. However, segregants could not be retested since the procedure did not allow survival of the animals. In each experiment, parental marrow cells were infused into both isogenic and F_1 hybrid recipients to provide independent evidence of donor cell viability as well as positive and negative controls of spleen repopulation.

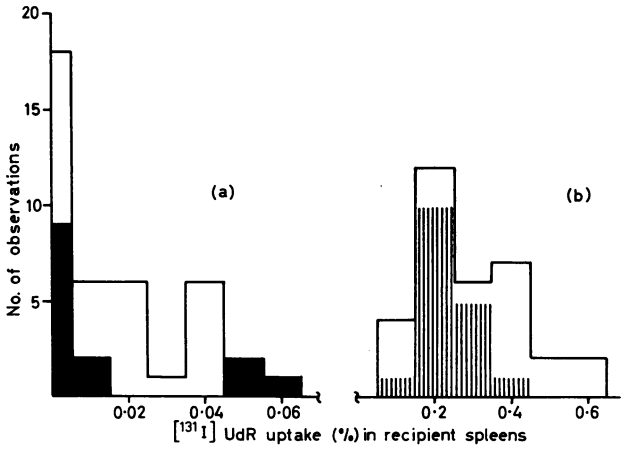

FIG. 4. Frequency distribution of [^{131}I] UdR spleen uptake in X-irradiated (700 R.) recipient mice infused with B/10 marrow cells. The data of two independent experiments were pooled. Thirty-seven (a) and thirty-three (b) observations in classes of segregating progeny. ||||, uptake in B/10 recipients (eighteen observations); ■, uptake in F_1-hybrid recipients (fourteen observations); □, uptake in backcross progeny (seventy observations).

Results obtained in 142 backcross-progeny mice are shown in Table 4. The data for the B/10 strain, which are also presented in the form of a frequency distribution diagram (Fig. 4), fit rather well the ratios to be expected if the trait were controlled by a pair of determinants at a single autosomal locus. The data for C3H and A donors indicate that these two strains do not share this determinant with B/10. In fact, spleen repopulation by C3H or A cells occurred uniformly in all their backcross-progeny tested. For this reason, it is not clear whether the alleles to the B/10 'resistance' factor, which should be present in the C3H and A strains, are alike or dissimilar.

Table 5 (Experiment 1) presents data on the segregation of the 'resistance' trait relative to three other loci at which the B/10 strain differs from the C3H and A strains. There was no apparent association with the Agouti and haemoglobin loci nor with sex, whereas 'resistance' segregated together with H-2 traits in sixty out of seventy animals tested.

TABLE 5

SEGREGATION OF 'RESISTANCE' RELATIVE TO OTHER LOCI

Locus	*Cross*	*Resistant*	*Susceptible*	*Resistant*	*Susceptible*	*Total*	χ^2	*P*
Agouti*	(C3H ×B/10)F_1 ×B/10	a/a		A/a		45	0·64	0·42
		12	8	12	13			
Haemoglobin*		Hb^s/Hb^s		Hb^d/Hb^s				
	(C3H ×B/10)F_1 ×B/10	4	1	6	3			
	(A ×B/10)F_1 ×B/10	6	7	7	5			
	Both crosses	10	8	13	8	39	0·9	0·34
H-2 and sex (Experiment 1)*		$H\text{-}2^b/H\text{-}2^b$		$H\text{-}2^k/H\text{-}2^b$ or $H\text{-}2^a/H\text{-}2^b$				
	(C3H ×B/10)F_1 ×B/10	3♀ 3♂	10♀ 10♂	5♀ 12♂	0♀ 2♂			
	(A ×B/10)F_1 ×B/10	1♀ 0♂	6♀ 4♂	5♀ 8♂	0♀ 1♂			
	Both crosses	7	30	30	3	70	36·3	< 0·001
H-2 (Experiment 2)†								
	($H\text{-}2^k/H\text{-}2^b$) ×$H\text{-}2^b/H\text{-}2^b$	0	9	17	0			
	($H\text{-}2^a/H\text{-}2^b$) ×$H\text{-}2^b/H\text{-}2^b$	0	14	13	0			
	Both crosses	0	23	30	0			

* Data from experiments presented in Table 4.

† Data obtained from additional backcross progeny; 700 R. of X-rays and 10^6 B/10 ♀ marrow cells.

Of the ten animals exceptional in this respect, seven were homozygous for the H-2^b allele but presumably heterozygous for the 'resistance' trait. These exceptions might be due either to errors in H-2 classification, technical failure of marrow transplantation, or to recombination. Perhaps part of them, if any, were H-2 misclassifications, for offspring from the backcross H-2^k/H-2^b × H-2^b/H-2^b were typed once by haemagglutination and only with antisera specific for the component K of the H-2^k allele. Similarly, backcross offspring from the mating H-2^a/H-2^b × H-2^b/H-2^b were also serotyped once for the components D and K of the H-2^a allele. The specificity determined by component K fails sometimes to be expressed on erythrocytes in amounts detectable by a single agglutination test, although repeated typing or progeny-testing would conclusively establish its presence or absence in the phenotype. The other three exceptional mice were supposedly H-2^k/H-2^b heterozygotes but 'resistance' homozygotes. They might be explained by errors in identification of mice, although this is unlikely, or by recombination.

To verify whether the relatively high frequency of exceptional animals encountered among backcross progeny could, in fact, be due to errors in H-2 classification, another group of fifty-three such animals were serotyped repeatedly using reagents anti C, K and H for backcross offspring of H-2^k/H-2^b heterozygotes, and reagents anti D, M, C, K and H for backcross offspring of H-2^a/H-2^b heterozygotes. The results (Table 5, experiment 2) indicated that twenty-three H-2 heterozygotes were resistant and thirty H-2^b homozygotes were susceptible to B/10 marrow grafts. H-2 traits and 'hybrid resistance' appear, therefore, to be controlled by the same or by linked loci; discrimination between these two alternatives can be attempted more efficiently if animals could survive typing for the 'resistance' trait so that progeny-testing and retyping of supposed recombinants would yield true values of recombination frequency.

DISCUSSION

The short-term transplantation data leave little doubt that the growth of marrow cells of two C57BL sublines was deficient in (C57BL × C3H)F_1 and in (A × C57BL)F_1 recipients. Similarly defective patterns of growth in F_1 recipients were described for C57BL antibody-forming cells (Boyse, 1959) and for leukaemias (Snell, 1958; Snell and Stevens, 1961). Popp (1961a) and Popp, Cosgrove and Popp (1964) reported that C57BL marrow is less efficient than 101 marrow in establishing permanent functional grafts in X-irradiated (C57BL × 101)F_1 mice, based on delayed disappearance of donor-derived erythrocytes (marked by haemoglobin characteristics) from the chimaera's peripheral blood. The reversions to host-type erythrocytes occurred with higher frequency in F_1 recipients of C57BL marrow than in such recipients of marrow from 101 donors or donors of other strains. However, similar reversions were reported in allogeneic radiation chimaeras of different constitution (Cudkowicz, 1963; Koller *et al.*, 1961, see VI, B). Furthermore, C57BL marrow is relatively inefficient in providing long-term grafts in F_1 hybrid and in allogeneic host animals (Popp *et al.*, 1964). Consequently, such regressions appear different in pathogenesis from the deficient growth of C57BL haemopoietic cells assessed 5–10 days after inoculation, which has been noted thus far only in parent-to-hybrid marrow transplantation (Table 3) and exclusively with donor marrow cells homozygous for the H-2^b allele (Cudkowicz and Stimpfling, 1963, and manuscript in preparation). The fate of long-term grafts might, for example, be affected by secondary changes of the immune reactivity of the transplanted marrow; e.g. development of specific unresponsive-

ness (Simonsen, 1960; Popp, 1961b). In addition, C57BL donor cells of long-term parent-to-hybrid chimaeras are highly selected in that only cells that override the 'hybrid resistance' repopulate the haemopoietic sites of the recipients.

Fox (1962) and Fox and Howard (1963) reported exceptionally good survival of C57BL spleen cells infused into (CBA × C57BL)F_1 mice. The data were obtained using a chromosomal marker for recognition of host-type cells and appear at first glance to contrast with the finding of 'hybrid resistance'. Reconciliation of the two sets of data is possible, however, (1) by regarding the number of donor cells used (10^8 cells per recipient) as largely sufficient to override the 'hybrid resistance', or alternatively (2) by postulating that their C57BL subline might be segregating for, or might have lost, the trait responsible for 'deficient growth in hybrids' (see later in the discussion).

Parental skin grafts are consistently tolerated in F_1 hosts, an indication that the 'hybrid resistance', when detectable, is relatively specific for parental haemopoietic cells and their neoplastic counterparts. These findings raise questions relevant to the general understanding of the biology of transplantation: do C57BL cellular grafts fail in F_1 hybrids because of their competence to initiate exhaustive graft-versus-host reactions, as proposed earlier by Boyse (1959) for antibody-forming cells, or is their failure dependent on histocompatibility-related growth requirements, specific for haemopoietic cell grafts as opposed to solid tissue grafts? Since these growth requirements are detectable in heterozygotes, are they determined by interallelic interaction or by recessive determinants?

The first question may be answered by the experiment in which the proliferative capacity of C57BL cells was assessed in C3H recipient mice. If excess of isoantigen exhausted the immunologically competent marrow cells to an extent that cell division was thereby suppressed, the isoantigens in C3H recipients should have been at least as detrimental to the proliferation of C57BL cells as the isoantigens in (C57BL × C3H)F_1 recipients. The data, on the contrary, demonstrated that inoculated C57BL marrow stem cells proliferated in heavily irradiated C3H mice in spite of excess of isoantigen, whereas proliferation of the C57BL cells in similarly irradiated hybrids remained suboptimal. However, their proliferation could be enhanced in F_1 recipients by increasing the X-ray exposure given before marrow infusion, a procedure which presumably should not have altered the antigenicity of the host tissues or the immunological competence of the donor cells. One can conclude, therefore, that the experimental evidence does not support the hypothesis that C57BL marrow cells were exhausted by isoantigen excess in the hybrid mice. Altogether, haemopoietic marrow from normal donors (i.e. from mice not exposed previously to isoantigenic stimulation) appears to contain few, if any, differentiated antibody-forming elements: runt disease, a manifestation of graft-versus-host reaction, can be minimized in newborn mice by injecting allogeneic marrow instead of spleen cells (Billingham and Brent, 1959). Accordingly, humoral antibody formation (Gengozian, Makinodan and Shekarchi, 1961) and foreign skin or tumour graft rejection (Koller and Doak, 1960; Bridges, Loutit and Micklem, 1960) does not occur in isogenic radiation-induced marrow chimaeras until 25–50 days after marrow infusion. The delay has been related to repopulation, differentiation and maturation in the host of undifferentiated marrow stem cells, since substitution of marrow by spleen cells or other cell suspensions containing differentiated antibody-forming elements results in reduction of such delay.

Little can be said at present about the nature of the 'hybrid resistance' to parental

C57BL cells; the hybrids are not 'resistant' to marrow cells from the C3H and A strains, although there is no evidence that the inherent capacity and the kinetics of cellular proliferation are different for C57BL and C3H marrow. The radiosensitivity of the 'hybrid resistance' to C57BL cells appeared to be definitely lower than that of C3H mice, an indication that the two phenomena might have different bases, in accordance with earlier conclusions (Snell, 1958; Snell and Stevens, 1961). Further work is required to elucidate this point; it is pertinent to mention here that it has been speculated (Hauschka and Furth, 1957; Snell, 1958; Stimpfling and Snell, 1962) that determinants other than the well-known H genes may influence graft success. The possibility that the F_1 hybrid is capable of reacting immunologically against parental haemopoietic tissue has been suggested by Cudkowicz (1961) and by Celada and Welshons (1962). The fate of C57BL cells transplanted into lethally irradiated F_1 hybrids pretreated with parental spleen cells was studied with methods involving survival and estimation of humoral antibodies derived from hyper-immunized donor spleen cells. Since the present report is confined to the study of C57BL marrow grafts in non-presensitized F_1 hybrids, no comparison can yet be made between the previous data and assessments of growth of marrow grafts obtainable by the use of the [^{131}I] UdR technique.

Occurrence of the 'hybrid resistance' in reciprocal F_1 crosses and in approximately half of the backcross progeny is a strong indication that success or failure of C57BL haemopoietic grafts was controlled in this system by a single pair of determinants at an autosomal locus. No resistance to C3H cells and A strain cells was observed in suitable backcross offspring. The fact that C57BL cells were able to proliferate in C3H mice is contrary to expectation, if a recessive gene hypothesis is assumed (Cudkowicz and Stimpfling, 1963). Linkage tests indicated that the genetic determinant under study was associated with, or part of, the complex H-2 region in the IXth linkage group. The region controls cellular isoantigens of primary importance for the fate of solid tissue grafts in the mouse. A clear distinction between association with the H-2 locus and control by the locus itself could not be made because of the relatively small number of segregants examined and limitations in the procedures followed for typing H-2 and 'resistance' traits. However, there may be no clear distinction since recombination within the H-2 region has been observed (Allen, 1955; Amos, Gorer and Mikulska, 1955; Pizarro, Hoecker, Rubinstein and Ramos, 1961; Stimpfling and Snell, 1962) and since the region also controls the synthesis of a specific mouse serum protein (Schreffler and Owen, 1963) the antigenic specificity of which is not in common with that of the H-2 cellular isoantigens. The available evidence strongly suggests that the deficient growth of C57BL cells in hybrids is associated with heterozygosity at the H-2 locus or at an independent H-2-linked locus. The possible role of heterozygosity at several other H loci has been excluded by experiments with hybrids from crosses between 'isogenic resistant' mice, differing at a single H locus with a common B/10 genome (Cudkowicz and Stimpfling, 1963, and manuscript in preparation). The occurrence of some form of allelic interaction involving H-2 or alternatively an H-2 linked locus is a plausible explanation, perhaps resulting in lack of expression of one or more C57BL strain isoantigens in the F_1 hybrids.

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